Molecular Probes for Rice Blast Disease

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A SMALL NUMBER OF FUNGAL PATHOGENS CAUSE SOME OF the most persistent and devastating crop diseases. Among these is an ancient disease of rice known as blast, which is caused by the filamentous fungus *Magnaporthe grisea* (*1*). Because rice is the major food source of many developing nations, the control of rice blast disease is important to world agriculture. However, because of the large number of virulence forms or pathotypes manifested by *M. grisea* (*2*), effective measures to control blast disease remain elusive. Although *M. grisea* has frustrated rice growers, work by Valent and colleagues has demonstrated that the fungus's haploid life cycle and simple growth requirements make it tractable for genetic and molecular analyses (*3*). Recent studies are now helping to resolve two long-standing and controversial issues in rice blast disease research.

First, the genetic relationship between isolates of *M. grisea* that infect rice and those that infect other grasses has not been clear. Although infections by *M. grisea* have been documented on over 30 different grasses, individual isolates are restricted to parasitizing one or two hosts. Historically, rice-infecting isolates and isolates that infect other grasses were considered different members of the same genus (*Pyricularia* spp.). The discovery of sexual compatibility between some of these isolates prompted their reclassification as a single species (*4*). A general absence of isozyme variation in *M. grisea* populations (*5*) as well as the occasional discovery of grass-infecting isolates that could also infect rice (*6*) similarly obscured the distinction between these two groups of pathogens. Consequently, it has not been clear whether isolates that infect feral grasses surrounding rice fields could be a potential source of rice blast disease.

The second issue concerns the diversity and pathogenic stability of rice-infecting *M. grisea* isolates. A standard epidemiological approach in plant pathology has been to use comparative infection assays, which involve a series of differentially resistant plant cultivars, to identify the pathotype (race) of a pathogen (7). However, pathotype assays of different blast fungus populations have produced conflicting results that have led to differing interpretations of pathotype diversity and genetic stability. Some researchers have suggested that hundreds of pathotypes may exist and that each isolate of a given pathotype may undergo sudden changes to a variety of other pathotypes (*8*). Others believe that there are a limited number of pathotypes and that the pathotype of an isolate is stable (*2, 9*). In areas where crops are prone to blast disease, the sudden infection of a previously resistant rice variety in the field is usually attributed to the appearance of one or more new races of *M. grisea*; new resistant rice varieties must therefore be introduced every few years. A clearer understanding of the epidemiology of the blast fungus would allow rice breeders and pathologists to make more judicious choices when breeding and selecting new resistant rice varieties.

An analysis of repeated DNA sequences in the *M. grisea* genome now provides a clearer picture of the genetics and evolution of host selectivity (*10, 11*). These same repeated sequences can also be used to locate pathogenicity genes on the *M. grisea* chromosome (*12, 13*). A family of *M. grisea* repeated sequences (MGR sequences) were found in a high copy number (approximately 40 to 50 copies per genome) in isolates that infect rice (*10*). *Magnaporthe grisea* isolates that do not infect rice contain relatively few copies of the MGR sequence. Thus, MGR-DNA sequences diagnostically mark populations of *M. grisea* that have a rice-specific pathogen genotype. The MGR-DNA sequence conservation demonstrates a monophyletic origin for isolates that cause rice blast disease and suggests that the rice blast pathogen has been distributed throughout the world along with its host. More importantly for rice farmers, these results suggest that *M. grisea* isolates that infect weeds near rice fields are unlikely to become sources of rice blast disease. This hypothesis was rigorously tested when *M. grisea* rice pathogens and weed pathogens obtained from the same locations in the Philippines were shown to be easily distinguishable on the basis of MGR sequence conservation (*14*).

MGR probes have also been used to examine the origins of blast disease on wheat, a disease that has been documented in Brazil since the early 1980s. Because of the prevalence of rice blast disease in the same locale, it was possible that the wheat pathogens had descended from the rice pathogens. MGR probes were used to analyze the DNA of *M. grisea* isolates that were obtained from a diseased area growing on wheat, rice, and feral grasses. The low number of MGR sequences in the DNA of the wheat blast isolates suggested that they were not closely related to the rice pathogens but were more likely derived from weed pathogens in the same area (*3, 15*). Although the phylogeny of the wheat blast isolates is not yet known, the Brazilian outbreak may offer a unique opportunity to capture a "snapshot" of the evolution of a new *M. grisea* crop pathogen.

An MGR probe has also been used to investigate pathotype variation and stability on a collection of U.S. blast fungus isolates (*11*). Blast disease occurs regularly in the rice-growing region along the U.S. Gulf Coast, and numerous blast fungus isolates were collected and stored from that region over a 30-year collection period. The MGR probes were used to construct genotype-specific profiles based on restriction fragment length polymorphisms (MGR-DNA fingerprints) of 42 isolates representing eight major blast fungus pathotypes found in the region. If pathotypes were unstable, there would be little correlation between DNA fingerprint and pathotype; hence, the clonal relatedness of isolates with a common pathotype would be obscured. These studies showed, however, that isolates of the same pathotype, collected from different areas at different times, had diagnostically similar MGR-DNA fingerprints (*11*). Thus, the major U.S. pathotypes were found to represent distinct genotypes composed of specific clonal lineages that were identified by consensus MGR-DNA fingerprints. Some pathotypes were found to be composed of more than one MGR-DNA fingerprint lineage, suggesting that episodes of convergent pathotype evolution can occur. In contrast, two pathotype groups were identified with highly similar MGR-DNA fingerprints, suggesting that both pathotypes were derived from a recent common ancestor. Thus the MGR probe was not only suitable for identifying pathotypes and distinguishing various isolates but could also be used to assess the phylogenetic relationships within and between various pathotype groups.

These results demonstrate that pathotypes of the blast fungus are not highly variable and that the population dynamics of this crop pathogen can now be reliably investigated. MGR-DNA fingerprinting can be used to assess the genetic diversity of the pathogen population in various rice-growing areas and to ensure that new crop cultivars are tested for resistance to all potential blast fungus

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genotypes found in a specific area. Perhaps more importantly, the MGR probes can also be used to document the phylogenetic histories of rice blast races in various parts of the world and to follow the mode and tempo of pathotype evolution. Molecular epidemiology studies of rice blast disease are now under way in rice-growing areas worldwide.

Genetic analysis of MGR sequences indicates that they are inherited in a predictable Mendelian fashion (*10, 12*). The genetic mapping of 48 MGR sequences suggests that they are randomly distributed about the genome and are useful as genetic markers in *M. grisea* (*16*). Although *M. grisea* is an ascomycete (like its famous cousin *Neurospora crassa*), field isolates, especially rice pathogens, show very poor fertility. Fertile laboratory strains were developed to facilitate genetic analysis and allow the genetic crossing of isolates with different host ranges (*3, 13*). A cross between a rice pathogen and a feral grass pathogen can now be used to follow the cosegregation of MGR sequences together with any other segregating phenotype, as is generally done for fragment length polymorphism markers. This genetic mapping approach was recently used to position a particular genetic locus—one involved in cell shape determination and pathogenicity—between two closely linked MGR sequences (*12*).

A recent report by Valent *et al.* (*13*) underscores the genetic differences between pathogens of rice and pathogens of other grasses and makes use of the MGR sequences in following complex genetic traits. In this study a strain obtained from a cross between a rice pathogen and a pathogen of weeping lovegrass was backcrossed to the rice pathogen parent. This backcrossing scheme was continued for several generations, and the progeny from each generation were scored for pathogenicity on three rice cultivars. Subsequent genetic analysis suggested that a small number of "major genes," inherited in a Mendelian fashion, appeared to govern an "all or none" ability to infect specific rice cultivars, whereas several "minor genes" appeared to affect lesion size on rice. MGR sequences were shown to accumulate in a sample of the backcross progeny, and linkage was detected between a particular MGR sequence and a gene that controls pathogenicity toward a particular rice cultivar.

MGR probes are now providing a clearer picture of the epidemiology and evolution of rice blast disease. In addition, they serve as useful genetic markers that could aid in the cloning of genes that determine host specificity and pathogenicity in *M. grisea*. However, the functional significance of the MGR sequences remains to be determined. The DNA sequence of one MGR has revealed an open reading frame with the potential to encode a reverse transcriptase with homology to the Long Interspersed Sequence (LINE) family of retrotransposons (*17*). Thus, MGR sequences may be capable of transposition and may play a role in shaping the *M. grisea* genome and in generating genetic diversity. Transposition or recombination events involving MGR sequences could affect the expression of genes that limit host specificity.

REFERENCES AND NOTES

1. S. H. Ou, in *Rice Diseases* (Commonwealth Agricultural Bureaux, Slough, U.K., 1985), p. 380.
2. One determines the pathotype, or race, of a rice blast fungus isolate by assaying its ability to infect a set of differential rice cultivars. The set generally includes seven or more cultivars that differ from one another by one or more resistance genes. Scoring of the pathotype assay requires a considerable amount of skill in deciphering infection severity over a range of lesion densities and lesion sizes. Difficulties in standardizing assay procedures and interpreting disease severity may lead to inflated estimates of race numbers (*9*).
3. B. Valent, *Phytopathology* **80**, 33 (1990).
4. M. E. Barr, *Mycologia* **69**, 952 (1977); A. Y. Rossman, R. J. Howard, B. Valent, *ibid.* **82**, 509 (1990).
5. H. Leung and P. H. Williams, *Phytopathology* **76**, 778 (1986).
6. A. O. Mackill and J. M. Bonman, *Plant Dis.* **70**, 125 (1986).
7. F. M. Latterell, in *Proceedings of the Seminar on Horizontal Resistance to the Blast Disease of Rice* (Centro International de Agricultura Tropical, Cali, Colombia, 1975), p. 167.
8. P. Giatgong and R. A. Frederiksen, *Phytopathology* **59**, 1152 (1969); S. H. Ou and M. R. Ayad, *ibid.* **58**, 179 (1968); S. H. Ou, *Annu. Rev. Phytopathol.* **18**, 167 (1980).
9. F. M. Latterell and A. E. Rossi, *Phytopathology* **76**, 231 (1986); J. M. Bonman *et al.*, *Plant Dis.* **71**, 127 (1987).
10. J. E. Hamer, L. Farrall, M. J. Orbach, B. Valent, F. G. Chumley, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9981 (1989).
11. M. Levy, J. Romao, M. A. Marchetti, J. E. Hamer, *Plant Cell* **3**, 95 (1991).
12. J. E. Hamer and S. Givan, *Mol. Gen. Genet.* **223**, 487 (1990).
13. B. Valent, L. Farrall, F. G. Chumley, *Genetics* **127**, 87 (1991).
14. R. Nelson *et al.*, in *Abstracts of the Fourth Annual Meeting of the Rockefeller Foundation's International Program on Rice Biotechnology* (Rockefeller Foundation, New York, 1990).
15. F. G. Chumley, L. Farrall, B. Valent, unpublished observations.
16. J. Romao and J. E. Hamer, unpublished observations.
17. M. J. Orbach, L. Farrall, F. G. Chumley, B. Valent, unpublished observations.